Cutibacterium acnes - It's Not Just a Problem for Teenagers: Improving Recovery in Rapid Sterility Validations

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Overview

With the validation and implementation of rapid respiratory-based sterility methods, a robust and proven methodology is needed that allows any analyst to generate reproducible recoveries of all required microorganisms. Experimentation was performed to develop a process that ensures consistent recovery for *Cutibacterium acnes*, a slow growing bacteria required for validating this test.

Background

Sterility testing is a required biosafety test for release of a sterile drug product. The organisms used in sterility method validation are recommended by various regulatory bodies (FDA, EMA) and documented in compendial guidance (USP, EP, JP.) The organisms encompass a wide range of potential contamination sources (human, environmental, water) to ensure patient safety.

In the context of method development testing for rapid sterility using the BACT/ALERT 3D at the Micro COE, the microorganism with the longest time to detection and the most variability is typically C. acnes. Therefore, the technique for inoculating the BacT bottles as well as the experimental parameters that could influence the recovery of fastidious microorganisms such as C. acnes was examined, and the potential impact on performance of the method in routine operation was considered.

Routine sterility test inoculation utilizes a safety transfer device to limit needlestick risk. Use of a safety transfer device eliminates variability in a number of inoculation parameters (e.g., depth of needle puncture, needle gauge, angle of puncture). Method validation uses traditional needles and syringes due to the need for greater operational flexibility during method validations.

BACT/ALERT 3D

The BACT/ALERT 3D is a system that utilizes normal metabolites (specifically CO_2) from viable microorganisms which change the pH of inoculated media. This change in pH is then detected by a photodiode which is then checked against an algorithm within the instrument to determine if the samples meet the criteria to be deemed positive for microbial growth.

Figure 1: BACT/ALERT 3D Dual-T System

Cutibacterium acnes

- Common human commensal organism
  - Mesophilic skin flora
  - Opportunistic human pathogen
- Gram-positive, aero-tolerant, rod-shaped bacteria
  - Tolerates oxygen but best growth under anaerobic conditions
- Temperature sensitive
- Slow grower
  - Generation times exceeds 5 hours, even at optimal conditions
  - Longest time to recovery/detection for respiratory based assays
- Common strains: ATCC 11827, ATCC 6919, DSM 1897

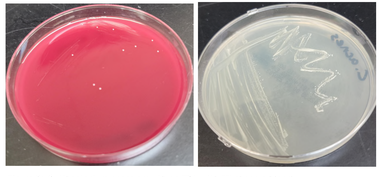

Figure 2: *Cutibacterium acnes growth on TSA with 5% sheep's blood (left) and TSA (right)*

Clostridium sporogenes

- Gram positive rod
- Strict anaerobe
- Fast grower compared to *Cutibacterium acnes*
  - Generation time approximately 45 minutes
- Human commensal of GI tract and environmental isolate (soil)
- Ideal microorganism for assessing anaerobic conditions of the environment
- Common strains: ATCC 11437, ATCC 19404

Experimental Design

Determine effect of various parameters to assess which produces the greatest improvement in microbial detection without introducing undue burden on analyst or assay.

Clostridium sporogenes was utilized for method and technique development due to its rapid growth rates and fastidious nature. Optimized conditions for *C. sporogenes* would then be subsequently leveraged to improve the growth and detection of *C. acnes*.

- Pre-treatment steps
  - Pre-warm rehydration fluids
- Inoculation techniques
  - Depth of puncture
  - Number of punctures (1 or 2)
  - Angle of insertion
  - Time and dispensing speed
  - Needle gauge (20 vs. 27 gauge)
  - Use of needlestick prevention devices
- Change incubation temperatures
  - Variations in temperature alter generation times

Results

Condition tested	What was tested?	Recovery rate	Conclusion
Pre-warm hydration fluid	Warm at 37C	1 of 12	Hydration fluid temperature has no effect
Needle gauge	20G vs 27G	2 of 24	Needle gauge has no effect
Depth of puncture and speed of dispensing	Deep puncture with vacuum/gravity withdrawing inoculum vs shallow puncture with rapid dispensing of inoculum	0 of 44 vs 43 of 44	Shallow puncture with rapid dispersal yielded nearly 100% recovery rate of a fastidious micorganism even at concentrations of <10 CFU even with 20G needle

Figure 3: High level overview of data generated during *C. sporogenes* method development

- Greatest improvement was with introduction of optimized inoculation technique
  - 29% Reduction in DTD (>6 Days to <5 Days)
  - Single, shallow puncture with rapid injection of material
  - Ensure angle of puncture is the same as entry
    - Movement can introduce ambient atmosphere which does not occur with the use of the safety device
- Improved recoveries with optimized temperatures
  - 5% Reduction in time to detection
    - Compounded with technique improvements

Internal Time to Detection Prior to Technique optimization	Average Time to Detection at 32.5C (Days)	Average Time to Detection 35C (Days)	Reduction with more optimized temperature against 32.5C	Reduction with optimized technique alone at 32.5C
6.10	4.45	4.23	5%	29%

Figure 4: Aggregate data comparing the days to detection for *C. acnes derived during initial internal testing against optimized technique and optimized technique and temperature*

Conclusion

- Improving analyst technique with inoculation of organisms can and will improve recoveries of difficult to culture organisms.
- Optimized technique mimics the safety device with regards to depth and angle of puncture.
- Needle gauge presents minimal risk and multiple gauges are acceptable for use in method development and routine operation.
- Product agnostic improvement due to inoculation technique of analyst improved recoveries for all microorganisms and lessened days to detection.
- Subsequent validations after adoption of technique demonstrated improved recoveries across multiple facilities and analysts with training.
- Continued vigilance related to method development and optimization is necessary to make small gains after initial observations of variability in detection times.
- Use of syringe and needles allows for flexibility and ease of use by analysts when conducting validation work or non-routine testing, but technique must be specific in order to ensure optimal, consistent recovery. Use of the safety devices in routine testing mitigates the risk.

References

- USP <71>, USP<72>, USP<1223>, USP<1071>, EP 2.6.1, EP 2.6.27, EP 5.1.6,

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